Effects of Cutting and Maturity on Antioxidant Activity of Fresh-Cut Tomatoes

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Keywords: minimally processed; radical scavenging capacity; $ABTS^{\cdot+}$; *Lycopersicum esculentum*, modelling

Abstract

To investigate the changes in total antioxidant activity of fresh-cut tomato during storage, tomato fruits harvested at three different stages of maturity were cut into 7-mm thick slices and stored at 5°C. Intact (control) fruits were stored in the same conditions. The antioxidant activity was evaluated as the capacity to scavenge the radical $ABTS^{\cdot+}$ in both hydrophilic (HAA) and lipophilic (LAA) extracts. Cutting resulted in a decrease in the HAA compared to control fruits and did not influence significantly the LAA. Changes in LAA during storage were described by a simple exponential model developing towards an asymptotic end value. The HAA also decreased exponentially in the beginning of the storage time but increased again afterwards. The hydrophilic antioxidant activity was higher than the lipophilic antioxidant activity for all stages of maturity. The levels of HAA did not differ significantly due to ripening during storage while the LAA increased with ripening.

INTRODUCTION

The consumption of a diet rich in fresh fruits and vegetables has been associated with a number of health benefits including the prevention of chronic diseases (WHO, 2003; Klerk et al., 1998). This beneficial effect is believed to be due, at least partially, to the action of antioxidant compounds, which reduce oxidative damage in the body. The changes that happen in fruits and vegetables during harvesting, handling and processing can affect antioxidant status (Lindley, 1998). Data on content and retention of bioactive compounds in minimally processed fruits and vegetables are sparse (Lindley, 1998). The generally accepted view, however, is that fresh cut tissues are primarily submitted to oxidative stress presumably causing membrane damage and altering the composition and content of antioxidant compounds resulting in changes in the total antioxidant activity of the tissue.

Decrease in the antioxidant activity after processing was reported for fresh-cut spinach (Gil et al., 1999) and fresh-cut mandarin (Piga et al., 2002). The content and composition of antioxidant compounds changed during the ripening of tomato fruits and this was reflected in changes in the antioxidant activity (Jimenez et al., 2002; Cano et al., 2003). If minimal processing operations result in changes in antioxidant activity, it is expected this will depend on the stage of ripeness of the fruit since the chemical composition of the fruit will be different. The objective of the present research was to investigate the changes in total antioxidant activity of fresh-cut tomato during storage.

MATERIALS AND METHODS

Chemicals

2,2'-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) as the crystallized diammonium salt was obtained from Biochemika. Horseradish peroxidase type VI (HRP), was obtained from Sigma. All other chemicals were of analytical or HPLC grade purity.

Harvesting, Processing and Storage

Tomatoes (cv. Belissimo) grown in a greenhouse in Made (The Netherlands) were harvested in a single day in April 2004 in three colour stages, corresponding to the following grades of the tomato colour chart (Kleur Stadia Tomaten- The Greenery-The Netherlands) : I = grade 3-4; II = grade 5; III= grade 9. After harvest, the fruits were sanitized with sodium hypochlorite solution (1 mg/l, pH 6.8) and sliced in 7-mm thick transversal slices. The four central slices were stacked in the same position they had in the fruit in a white polystyrene tray (138 mm x 138 mm x 25 mm), covered with a plastic film (Magnetron) and stored at 5 ± 0.5^{o}C. Intact fruits stored at the same conditions were used as controls. For each maturity x temperature x storage time combination, 5 replicates, corresponding to a tray with 3 fruits were used. Antioxidant activity was determined every other day, starting one day after processing, in a total of 6 evaluations.

Extraction

At the indicated intervals, the slices and intact fruits were removed from storage. Extraction was based on the procedure described by (Arnao et al., 2001), using an exact weight of tomato (around 1g), mixed with 3 ml of Na-phosphate buffer (pH 7.5) and 5 ml of ethyl acetate. The homogenate was centrifuged two times, first at 6000 rpm for 5 minutes at 5^{o}C (Hettich-Universal 16R) and later at 16.400 rpm for 5 min at 5^{o}C (Eppendorf centrifuge 5417R). The hydrophilic extract was analysed immediately. The lipophilic extract was stored at –80 ^{o}C overnight and analysed in the next morning.

Antioxidant Activity Assay

Antioxidant activity was measured using a modified version of the ABTS/HRP decolouration method as described in (Arnao et al., 2001). For the hydrophilic antioxidant activity (HAA), the reaction mixture contained 2 mM ABTS, 20 nM HPR, 60 μM H_2O_2, in 50 mM Na-phosphate buffer (pH 7.5) in a total volume of 2 ml. For lipophilic antioxidant activity (LAA) the reaction medium contained 0.7 mM ABTS, 200 nM HRP and 60 μM H_2O_2, in pure ethanol acidified with phosphoric acid (0.6 μl/ml) in a total volume of 2 ml. Both reactions were monitored at 730 nm for 1 minute to check whether the absorbance was stable. Then, 60 μl of the aqueous extract or of the ethyl acetate extract was added to the reaction medium and the decrease in absorbance, which is proportional to the amount of $ABTS^{\cdot+}$ quenched, was determined at intervals of 1 minute, for 5 minutes. The absorbance decrease was determined from the difference between the A_{730} values before and 5 min after sample addition. Antioxidant activity was calculated as moles of $ABTS^{\cdot+}$ quenched by 1 mol of Trolox, based on the stœchiometric relationship that 1 mol of ABTS quenches 2 mol of Trolox (Arnao et al., 2001). Spectrophotometric measurements were recorded a USB 2000 Fiber Optic Spectrometer interfaced on line with a PC-computer.

Statistical Analysis

Data were first analysed by Analysis of Variance using PROC GLM from SAS (Statistical Analysis System) software considering stage of maturity, treatment (processing or not) and storage time as sources of variance. Later a non-linear regression analysis was conducted in order to study the changes in time, taking those factors and interactions into consideration that were significant in the analysis of variance. The model was developed as described in the next section and calibrated using the nonlinear regression procedure in GENSTAT (Rothamsted, UK).

Model Development

The changes in the antioxidant activity (AA) of fresh-cut tomato slices was described and analysed with a simple exponential model (first order kinetics) developing towards an asymptotic end value. The AA was considered to be built up by a variable part that changes according to a first order mechanism and a fixed part that is invariable or is in steady state at the circumstances under study.

This resulted in the basic first order model as described in Eq. 1:

$$AA = (AA_0 - AA_{fix}) \cdot e^{-k \cdot t} + AA_{fix} \quad \text{Eq. 1}$$

Where:

AA= antioxidant activity at time t after harvest

AA_0= initial antioxidant activity at harvest

AA_{fix}= invariable part of the antioxidant activity

k= reaction rate constant (at storage temperatures)

t= time (in days), counting from the moment of harvest.

In the present experiment, the tomato fruit were harvested from the same greenhouse at the same growing conditions but at different stages of ripeness. Unlike the development of colour (Lana et al., 2004) and firmness (Lana et al., 2005), the level of antioxidants apparently starts to decay only after harvest (disruption of the steady state at growing conditions), especially after processing (induced wound response). The hydrophilic antioxidant activity increased again at the later stages of storage. A linear increase was therefore added to the simple exponential model (Eq. 2). The increase in activity at the later stages of storage was dependent on the treatment. A separate value of reaction rate k_l was therefore allowed for both treatments. To accommodate for the differences in the general level of antioxidant activity between the treatments (cut-whole) and the stage of development (I, II, III) the asymptotic end value was made dependent on these factors.

$$AA = (AA_0 - AA_{fix}) \cdot e^{-k \cdot t} + AA_{fix} + \Delta AA_{stage} + \Delta AA_{treat} + k_{l,treat} \cdot t \quad \text{Eq. 2}$$

Where:

ΔAA_{stage}=shift in AA depending on the stage of ripeness

ΔAA_{treat}=shift in AA depending on the treatment

$k_{l,treat}$= rate constant for the linear increase in AA (at storage temperatures), depending on the treatment applied

Data Analysis

Based on equation 2, a non-linear regression analysis was performed (Genstat Rothamsted, UK). The data averaged over the 5 replicates were analysed without further transformation using ripening stage, treatment (cut or whole fruit) and time simultaneously as explaining variables (multi-variate non-linear regression analysis). The kinetic parameter (k), the invariable part of AA_{fix} and the initial value of AA_0 were estimated in common for all the slices. All other parameters were estimated separately for each stage of maturity of treatment.

RESULTS AND DISCUSSION

Analysis of Variance

The results obtained for antioxidant activity using the ABTS assay are presented in Figure 1 and in Figure 2. Minimal processing decreased the HAA ($Pr > F = < 0.0001$) but had no effect on the LAA ($Pr > F = 0.1660$) (Table 1).

Changes in LAA during storage were dependent on the stage of maturity of the fruit while the changes in HAA depended both on processing and on fruit maturity stage (Table 1). A traditional approach to study the changes in antioxidant activity on time would involve the decomposition of the interactions stage x time and treat x time for HAA and stage x time for LAA, that is the study of the effect of treatment and stage of maturity for each sampling date. Using this approach and given the fact that the sampling time is different in the individual series, makes it difficult to obtain a more general picture of the behaviour in time and how it depends on the other factors under study (in the present case stage of maturity and processing). Instead, the data were analysed using non-linear regression.

Modelling

The first regression analysis was conducted for each treatment (cut and intact) separately (Eq. 1). Later, the effect of treatment was incorporated (Eq. 2) so that the combined analysis included simultaneously the effect of time, treatment and stage. Non-linear regression is an iterative procedure and therefore strongly relies on good initial values for the parameters to be estimated. These values were obtained in a sequence of analyses, each step increasing in complexity in relation to the previous one, while building up the number of reliable estimates. Even in the final analysis (see results Table 2) not every parameter could be estimated simultaneously. This is the case, for example, of AA_0 for LAA. The reason is that in the time range where a rapid decay occurs, too few measuring points (about 3) were taken. That made it impossible to estimate the initial value and the asymptotic end value in the same analysis.

The antioxidant activity of both the hydrophilic and lipophilic extract showed the same exponential decay starting after processing, with a reference rate constant of 0.949 ± 0.091 for LAA and 0.281 ± 0.065 for HAA. Later on, the HAA increased with storage time, while the LAA remained practically unchanged. The estimated values for the rate constant of increase ($k_{l,treat}$) where consequently significantly positive for HAA (Table 3) while close to zero for LAA (not shown). Changes in LAA therefore could well be explained by Eq. 1, without the incorporation of the linear increase. The scatterplot for LAA (Fig. 3) and for HAA (Fig. 4) indicates a good fit of the model to the data.

The pattern of change of HAA during storage was the same whether the fruits were intact or cut, but the antioxidant activity was systematically lower for cut fruits (negative value for ΔAA_{cut} with ΔAA_{whole} fixed to zero). A similar pattern of change was reported for fresh-cut mandarin where the antioxidant activity decreased in the beginning of the storage period and later increased (Piga et al., 2002). This was considered to be due to changes in the antioxidant activity of polyphenols, which when undergoing enzymatic or chemical oxidation exhibit increased antioxidant efficiency in an intermediary state. The rate constant for this linear increase depended on treatment (Table 2) and was higher for intact fruits. This indicates that the significance of the interaction treatment x time was mainly related to differences in the magnitude of the linear increase, since the reaction rate for the exponential decay could be estimated in common for both cut and intact fruit.

Significant changes in LAA were only observed in the first three days of storage but could not be ascribed to cutting since the same phenomenon occurred in intact fruits. The estimated value of $\Delta AA_{cut,}$ although negative, was rather small and could not be estimated reliably (Table 2). The reasons for this decrease in LAA could not be determined in the present work. Probably the physiological response to the stress induced by harvesting consumed a major part of the available antioxidant activity. Reports on the lipophilic antioxidant activity as a response to cutting are unknown by the authors.

The radical scavenging capacity increased with ripening for both fractions lipophilic and hydrophilic. The increase in LAA with ripening was also reported by Cano et al. (2003), and related to changes in lycopene content. Contrary to those authors, a significant increase in HAA with ripening was found in the present work. The higher values for the estimates ΔAA_{II} and ΔAA_{III} for LAA, indicate that the effect of stage of maturity was more pronounced in LAA than HAA confirming the information obtained from the ANOVA where F values of 177.76 and 49.27 were obtained respectively for LAA and HAA.

The same pattern of changes during storage was observed for all stages. That means that differences between fruits at different stages during storage were mainly due to differences in the initial antioxidant activity when the fruits were processed.

Significance of the Decrease in HAA

The present results indicate that processing can induce a reduction of radical scavenging capacity, which is only one of the mechanisms by which antioxidants can prevent oxidative damage. The fact that an artificial radical was used should be kept in

mind when extrapolating the results to an in vivo situation, since the tested antioxidant may not present the same activity against physiologically relevant radicals.

Although a minor decrease in antioxidant activity was measured as a response to processing, at the moment it is not possible to assess the impact of this decrease in the value of the food as source of antioxidant in the diet. In spite of the agreement about the health benefits of antioxidants, there is no dietary prescription of recommended daily consumption as it is the case for nutrients such as vitamins and proteins.

CONCLUSIONS

The processing of tomato fruits into slices induced a decrease in the hydrophilic antioxidant activity compared to intact fruits stored at the same conditions. These changes were dependent on the stage of maturity of the fruit at harvest. The lipophilic antioxidant activity decreased exponentially towards an asymptotic end value during storage for both cut and intact fruits, without a significant effect of processing. The initial hydrophilic and lipophilic antioxidant activity were higher the more mature the fruit. Information obtained from analysis of variance can effectively be used in developing dynamic models.

ACKNOWLEDGMENTS

The authors are grateful to Dr. Marino Arnao and Dr. Antonio Cano from University of Murcia, Spain for their technical assistance in the ABTS assay. This work was supported by CNPq-Conselho Nacional de Desenvolvimento Científico e Tecnológico, Brazil.

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Tables

Table 1. Results of analysis of variance for the lipophilic (LAA) and hydrophilic (HAA) antioxidant activity of sliced tomato.

Extract	Source of Variation	F value	Pr > F
LAA	Stage of Maturity	177.76	< 0.0001
	Treatment	1.94	0.1660
	Storage Time	90.87	< 0.0001
	Stage * Treatment	0.19	0.8299
	Stage * Time	2.37	0.0125
	Treatment * Time	1.75	0.1263
	Stage * Treatment * Time	1.19	0.3006
HAA	Stage of Maturity	49.27	< 0.0001
	Treatment	174.14	< 0.0001
	Storage Time	20.15	< 0.0001
	Stage * Treatment	2.67	0.0732
	Stage * Time	2.79	0.0035
	Treatment * Time	3.73	0.0034
	Stage * Treatment * Time	1.48	0.1514

Table 2. Result of statistical non-linear regression based on Eq. 1 for LAA (lipophilic antioxidant activity) and Eq.2 for HAA (hydrophilic antioxidant activity).

	LAA		HAA	
Parameter	Estimate	s.e.	Estimate	s.e.
ΔAA_I	0	Fixed	0	Fixed
ΔAA_{II}	5.87	1.26	2.34	1.51
ΔAA_{III}	13.38	1.26	9.15	1.51
k	0.949	0.091	0.281	0.066
AA_0	40.0	Fixed	40.09	6.55
AAA_{fix}	29.16	1.09	55.83	3.69
ΔAA_{whole}	0	Fixed	0	Fixed
ΔAA_{cut}	-0.81	1.03	-5.06	2.49
$k_{l,whole}$	-		3.000	0.282
$k_{l,cut}$	-		2.106	0.282
N obs	36		36	
R^2_{adj}	87.5		80.7	

Figures

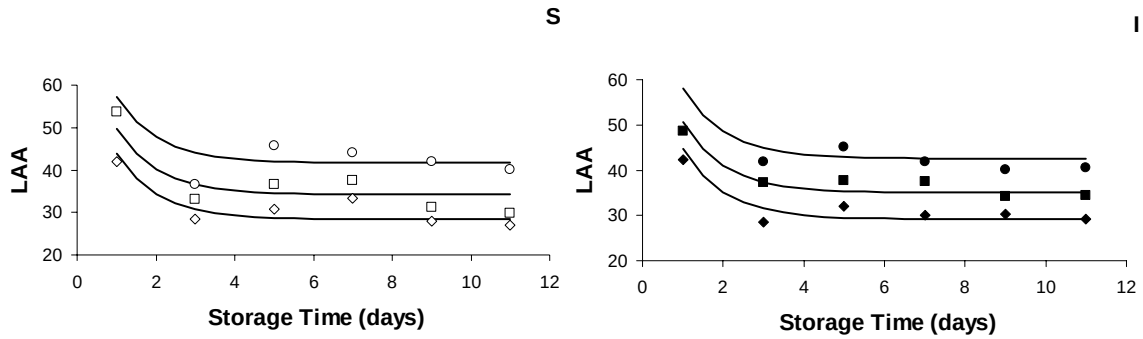

Fig. 1. Lipophilic antioxidant activity of tomato fruits harvested at stages I (♦), II (■) or III (●) and stored sliced (S) (open symbols) or intact (I) (closed symbols) at 5°C. Points are measured data (means of 5 replicates) and lines are simulated values according to Eq. 1.

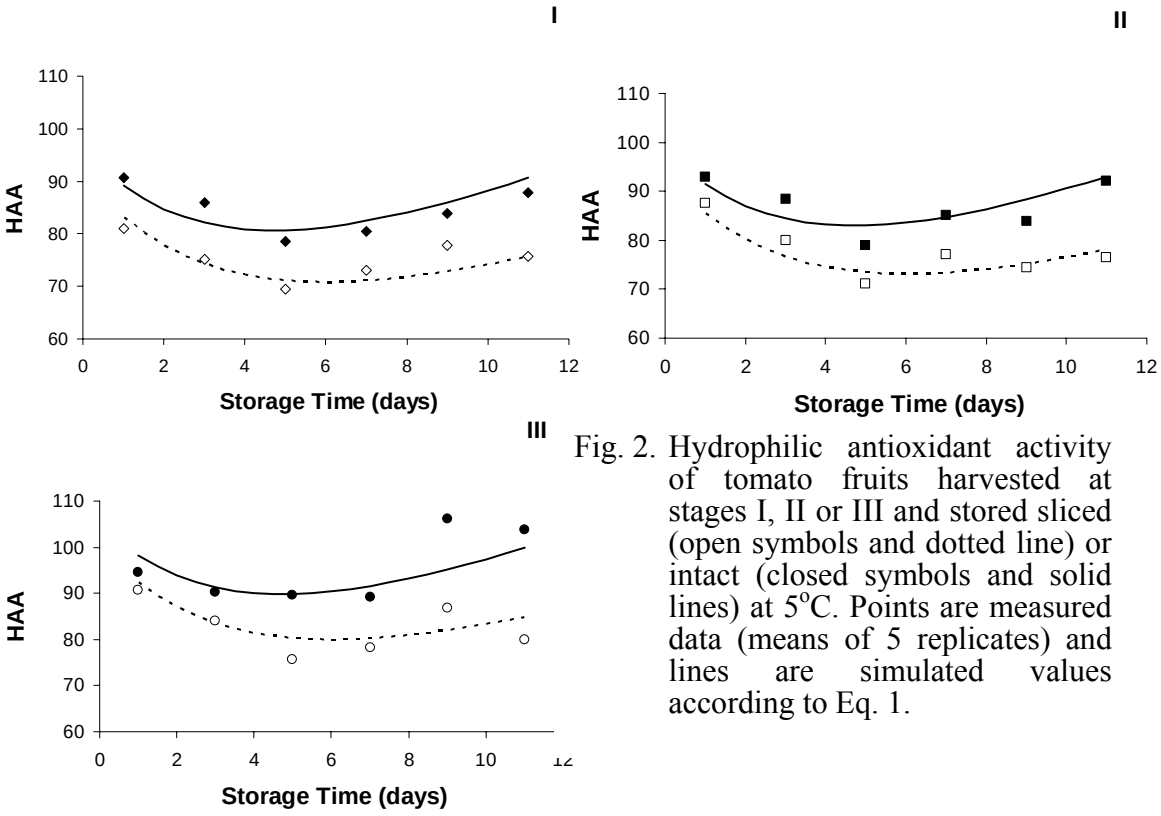

Fig. 2. Hydrophilic antioxidant activity of tomato fruits harvested at stages I, II or III and stored sliced (open symbols and dotted line) or intact (closed symbols and solid lines) at 5°C. Points are measured data (means of 5 replicates) and lines are simulated values according to Eq. 1.

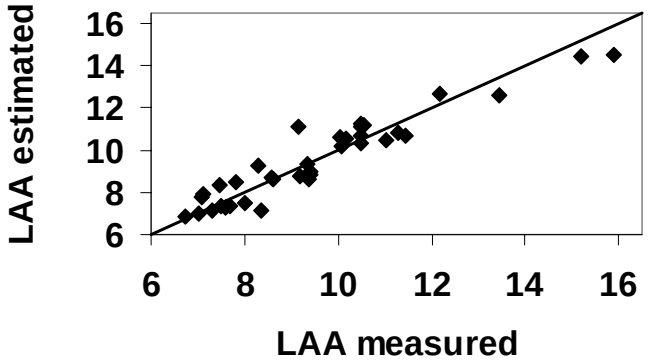

Fig. 3. Scatter plot for mean data of lipophilic antioxidant activity.

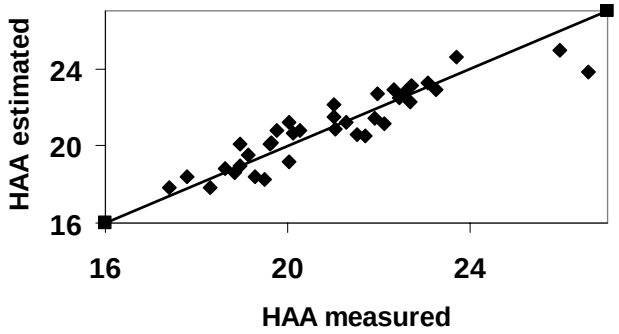

Fig. 4. Scatter plot for mean data of hydrophilic antioxidant activity.